REMARKS

Status of the Claims

Claims 1-4, 6-10, and 12 are currently pending. Claims 6, 7, and 12 have been amended as shown above. Claim 8 has been canceled. Claim 13 has been added. No new matter has been added by way of these amendments. After entry of the present amendments, claims 1-4, 6-7, 9-10, and 12-13 will be pending.

Withdrawal of Claim Objection and Rejections

Applicants appreciate the withdrawal of the objection to claims 5-12 and the rejection of claims 1-4 under 35 U.S.C. § 112, first and second paragraphs, for the use of certain terms.

Rejection under 35 U.S.C. § 112, Second Paragraph

Claim 12 remains rejected as allegedly indefinite for recitation of the term "controlling." Although the Examiner argues the interpretation advanced by the applicants is unsupported by standard medical dictionaries, such as Stedman's, the applicants note that the portion of Stedman's presented by the Examiner does define "control" as "regulation or maintenance of a function, action, reflex, etc." Thus, one of skill in the art would readily understand that "controlling" a viral infection means to "control" or "regulate" viral growth and/or replication so as to "maintain" viral load.

Nevertheless, to advance prosecution, claim 12 has been amended to replace the term "controlling" with the term "treating." In the applicants' view, treating a viral infection by definition includes not only treating symptoms of viral disease once they appear, but controlling the growth and/or replication of the virus so that an already infected patient does not become symptomatic. Applicants request withdrawal of this rejection in view of the amendments and arguments presented herein.

Rejections under 35 U.S.C. § 112, First Paragraph

Claims 6-8 and 12 are rejected as allegedly lacking enablement for the full scope of the claims. Claim 8 has been canceled.

With respect to claim 6, which is directed to a method for preparing a medicament, the applicants note that claims 9-10 are directed to the medicament itself (without a limitation on use) and have not been rejected. The language of claim 6 has therefore been amended to align more closely with claims 9-10. Applicants respectfully request withdrawal of the rejection of claim 6.

With respect to claims 7 and 12, the Examiner notes the claims are directed to viral infections broadly (Office Action, pages 5-6); these two claims have been amended to limit the viral infections to those arising from HCMV or another member of the group herpes viridae. This amendment should overcome the Examiner's concern in this regard.

The applicants have demonstrated, with supporting data, that the compounds of the instant application show an anti-HCMV activity; claims 7 and 12 are therefore enabled with respect to treatment of an HCMV or related viral infection. In view of the disclosure of the compounds, method of making the compounds, and methods of testing the compounds, it would not entail undue experimentation to assess the activity of the compounds recited in claims 7 and 12. That certain of the compounds within the broad genus may not display optimal activity does not undermine the enablement of the claims.

Attached hereto is a paper published by the applicants that demonstrates that a structurally related compound is indeed effective in the treatment of human patients with HCMV. (Zimmermann et al., European Infections Disease, 2011, 112-114.) Notably, the Examiner referred to the publication in which this compound was disclosed, WO 2004/072048, as the "closest prior art of record." This paper also shows that the related compound was effective in treating both infected patients in a Phase II trial and patients with established, refractory, CMV disease (see page 114), in contrast to the Examiner's comments that the literature did not support "treating" for late-stage CMV patients (Office Action, page 7).

The Examiner argues that the binding data and graft model disclosed in the present application provide insufficient correlation with actual treatment (Office Action, pages 6-9), but the Zimmermann paper shows that the related compound performed well in both the xenograft model (page 113) as well as in clinical settings (page 114). These data provide strong support for the use of the binding and graft models as suitable measures for methods of treatment.

The Examiner also rejects claims 6-8 and 12 on the basis that "prophylaxis" of viral infections is not enabled (Office Action at page 6, 10). This language has been removed from claim 6 and claim 8 has been canceled. In support of claims 7 and 12, enclosed please find a copy of a paper on antiviral strategies to combat cytomegalovirus infections in transplant patients, published by Lischka and Zimmermann in Current Opinion in Pharmacology. This paper makes it quite clear that, despite how the term "prophylaxis" might be understood in other fields, in the context of combating cytomegalovirus infections, the term "prophylaxis" means the "pre-emptive" treatment of patients at risk of developing HCMV disease with antiviral drugs, *e.g.,* from the time of the transplantation onwards (see page 3, left col., 4th para. to page 4, left col., 1st para.). See also, Zimmermann, European Infectious Disease, at page 114 (Phase II data). The goal of such treatment is not to prevent infection itself, but to keep the viral load down so that already infected patients do not progress to a symptomatic disease state. Therefore, the term "prophylaxis" in the present claims is a well-defined and understood term for the person skilled in the art of combating cytomegalovirus infections.

Importantly, it is generally accepted that most if not all currently available anti-HCMV drugs could be used in a prophylactic way with some suffering from toxicity profiles that make them unsuitable for long-term use (see Lischka, at pages 3-4, noting that, "prophylactic...strategies have been used with currently approved drugs....," and concluding that "[o]nce better tolerated drugs become available, treatment guidelines will likely favour prophylactic therapy...."). As discussed above, the compounds of the instant application combat HCMV by inhibiting the viral terminase complex. This target does not exist in humans and, therefore, it is to be expected that the compounds of the instant application should show a low interaction with non-targeted aspects of human metabolism. As a result, the present compounds enjoy a high safety margin, which is stated as one of the requirements for HMCV drugs to be used in prophylactic therapy (see Lischka, at page 3, Table 1). On this basis, a person skilled in the art would be aware that the compounds of the instant application would be suitable for prophylactic use.

Finally, the applicants have tested a compound that acts by the same mechanism of action as the compounds of the instant application, *i.e.*, another terminase inhibitor, whereby the cells were contacted with test compounds either before or after being contacted with HCMV. In <u>both</u> cases,

the terminase inhibitors showed a similar level of antiviral activity, again indicating that terminase inhibitors generally are useful in a prophylactic scenario.

The prophylactic viability of anti-HCMV compounds was confirmed by performing the above experiment with the commercially available compound ganciclovir. Like the terminase inhibitor, ganciclovir showed activity in <u>both</u> test scenarios. Notably, the tested terminase inhibitor was distinctly more active than ganciclovir in this regard.

At page 8 of the Office Action, the Examiner states that there is no guidance in the specification regarding how to select patients, compounds, viral infections, dosages or treatment regimens for prophylactic therapy. First, the specification itself does provide instruction as to specific patient populations that would benefit from prophylactic treatment. Zimmermann provides additional evidence that one of skill in the art would have understood that immuno-compromised patients with positive CMV viremia would be examples of suitable subjects. Thus, it would be well within the skill in the art to select suitable patients for this therapy. As discussed above, the specification teaches how to make and test the compounds of the invention. Finally, determination of specific dosages or treatment regimens would be within the skill of a treating physician.

In view of all this, it is quite clear that the person skilled in the particular art relevant to the present application would be familiar with the term "prophylaxis," would know how to use the compounds of the instant invention in a prophylactic scenario, and would reasonably expect the recited compounds to show the claimed prophylactic effects. In summary, the applicants request withdrawal of the rejection of claims 7 and 12 based on an alleged lack of enablement for prophylaxis.

<u>Conclusion</u>

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark Office determines an extension and/or other relief is required, the applicants petition for any required relief including extensions of time and authorize the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing Docket No. 584212009400. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

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Respectfully submitted,

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Current Opinion in Pharmacology

Antiviral strategies to combat cytomegalovirus infections in transplant recipients

Peter Lischka and Holger Zimmermann

Human cytomegalovirus remains an important pathogen for transplant recipients. To date, a limited number of drugs have been licensed for the treatment of HCMV infection and disease, all sharing the same target molecule, the viral DNA-polymerase. Although combating HCMV with DNA-polymerase inhibitors is effective and has been established for many years, there are several drawbacks associated with the use of these drugs including toxicity and emergence of drug resistance. In order to overcome these problems different treatment options and durations have been assessed and new and improved antiviral drugs with novel molecular targets have been discovered. However, not all of these novel drugs had the properties for success in clinical development, and alternative treatment options with known drugs have been evaluated in parallel. Today the need for an antiviral drug that is potent, safe and well tolerated remains.

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Introduction

Human cytomegalovirus (HCMV)

HCMV, the prototypic *Betaherpesvirus*, is an enveloped, double-stranded DNA virus that is spread through close contact with infected body fluids. Seroprevalence rates vary widely from 40% up to almost 100% depending on socio-economic status and geographic location. As with other herpesviruses, a primary HCMV infection is followed by the establishment of a life long latency in the infected host. HCMV rarely causes clinical manifestations in healthy individuals. However, a primary infection or reactivation of endogenous virus in immunocompromised individuals, such as transplant recipients, is associated with serious and life-threatening diseases. Although effective drugs to combat HCMV infections are available, they are associated with toxicity issues and emergence of drug resistance. Hence, new and improved antivirals with novel targets are urgently needed [1,2••].

HCMV pathogenesis in transplant recipients

Worldwide more than 130 000 solid organ and bone marrow transplants are performed annually, and HCMV continues to be a major cause of morbidity and mortality following both solid organ transplantation (SOT) and bone marrow transplantation (BMT) owing to a combination of direct cytopathic effects of replication and indirect, host dependent immunopathological mechanisms. The most common direct effects are HCMV syndrome (fever, anorexia, malaise), gastrointestinal disease, retinitis and hepatitis, whereas indirect effects of HCMV infection include allograft failure, restinosis and arteriosclerosis and a higher predisposition to opportunistic infections. HCMV infections observed in transplant recipients result either from reactivation of latent virus in HCMV-seropositive patients (R+) or from transmission of a primary infection from seropositive donor (D+) to a seronegative recipient (R−). The estimated incidence of HCMV disease in the absence of antiviral intervention depends on the transplanted organ. Symptomatic HCMV infection occurs in 8–32% of kidney transplant recipients, 22–29% of liver transplant recipients, 39–41% of lung/heart–lung transplant recipients and in almost 50% of pancreas transplant recipients [3–5]. The risk of HCMV disease following allogenic BMT is 20–35% in HCMV seropositive graft recipients. The most common clinical manifestations in this setting are enteritis and severe pneumonia. In general BMT transplant patients are at greatest risk for HCMV disease within the first three months post-transplantation [6,7]. However, a significant proportion of transplant recipients may develop HCMV disease within two years after transplantation. Importantly, although antiviral therapy has reduced the occurrence of early HCMV infection, the late onset HCMV disease is increasingly recognized in transplant patients of all kind [8,9].

Current antiviral therapy

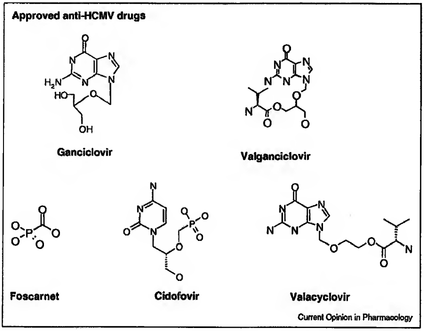
Antiviral drugs marketed to date

To date a limited number of drugs have been licensed for the treatment of HCMV infection and disease (Figure 1) [10].

Ganciclovir (GCV; Cymeven®, Roche)/Valganciclovir (VGCV; Valcyte®; Roche).

The current treatment of choice for HCMV associated disease in transplant patients is VGCV, the oral prodrug of

Figure 1



Chemical structures of marketed anti-HCMV drugs administered to transplant recipients.

GCV. VGCV, the L-valyl ester of GCV has been developed to overcome the low oral bioavailability of GCV and has replaced oral GCV in clinical practice [11,12]. GCV is a deoxyguanosine analogue that is selectively monophosphorylated in HCMV infected cells by the virus' encoded protein kinase pUL97. Subsequently, GCV is converted to the triphosphate by cellular enzymes. This active form preferentially inhibits the viral DNA polymerase. GCV is also incorporated into the viral DNA and drastically slows down chain elongation [13,14]. Neutropenia, anaemia, thrombocytopenia and a putative long-term reproductive toxicity are the most common serious side effects associated with GCV treatment [15•].

Cidofovir (CDV; Vistide®, Gilead)

CDV is a phosphonomethoxy analogue of cytosine that has demonstrated broad-spectrum activity against double-stranded DNA viruses including herpesviruses. In contrast to GCV, CDV does not require initial modification by a viral enzyme, although it is converted to its triphosphate by cellular enzymes. The active form of CDV acts as a competitive inhibitor of the viral DNA polymerase and causes premature chain termination during viral DNA synthesis [16]. CDV is highly active against HCMV and is characterized by a long intracellular half-life allowing infrequent administration of the drug. However, its clinical usefulness is limited since the drug is slowly absorbed, poorly bioavailable and causes severe nephrotoxicity. Moreover, cidofovir is carcinogenic and teratogenic in animal studies thus rendering it a second line therapy [17,18].

Foscarnet (FOS; Foscavir®, Astra-Zeneca)

FOS is a pyrophosphate analogue that directly binds to the pyrophosphate binding site of the viral DNA polymerase. The drug inhibits the DNA replication by blocking the cleavage of the pyrophosphate group from the terminal nucleoside triphosphate added to the growing DNA chain. FOS does not require any intracellular chemical modification for antiviral activity [19]. The most important major adverse effect associated with FOS therapy is renal impairment. Owing to this unfavourable toxicity profile and the fact that the drug must be given i.v. two to three times a day, FOS is also reserved for second line therapy. Nevertheless, FOS is the preferred rescue medication for patients that have failed GCV therapy [20].

Formivirsen (ISIS 2922; Vitravene®, Isis Pharmaceuticals)

Formivirsen is a 21 bp antisense oligonucleotide (5′-GCG-TTT-GCT-CTT-CTT-CTT-GCG-3′) that specifically targets the mRNA of the essential HCMV immediate-early-protein IE2 thereby inhibiting its translation. Formivirsen has been approved specifically for patients with HCMV retinitis, but due to its size and charge, it cannot penetrate tissues and must be administered by intraocular injection. Adverse events associated with Fomivirsen treatment are intraocular pressure and intraocular inflammation

[21]. Although highly innovative as the first antisense therapy reaching the market, the drug was voluntarily withdrawn from the European market for commercial reasons in 2002 but is still available in the US.

Acyclovir/Valacyclovir (VACV, Valtrex®, GlaxoSmithKline)
Although Aciclovir or its oral prodrug VACV have primarily been developed for the suppression of Herpes Simplex Virus (HSV), prophylactic use of VACV also has the potency to reduce the incidence of HCMV disease [22]. The nucleoside analog ACV (Figure 1a) has a mechanism of action similar to GCV and displays an excellent safety profile. However, VACV lacks sufficient potency to be used for the treatment of active infection, and VACV has therefore only been approved for the prophylaxis of HCMV infections in SOT transplant patients and in a limited number of countries.

Given the side effect issues of the available effective drugs on the one hand and a highly vulnerable patient population on the other hand, different treatment options and durations have been assessed in order to achieve an optimal outcome.

Intervention strategies: prophylactic vs. pre-emptive
There are two strategies to combat HCMV: prophylaxis and pre-emptive therapy. In prophylaxis the antiviral drug is given to all patients at risk from the time of transplantion onwards. In pre-emptive therapy patients are monitored by means of laboratory tests for active HCMV infection and are only treated once a certain HCMV threshold has been detected (Table 1).

Usage of drugs and treatment guidelines are rather complex. Recommendations differ remarkably depending on geographic region and type of transplantation. Moreover, in practice HCMV medications are frequently used off label. Although both strategies have been successful in controlling HCMV end-organ disease and indirect effects (including graft rejection) certain differences can be seen [23••,24••,25]. Non-randomized clinical trials may suggest that late onset disease is more problematic with prophylaxis than with pre-emptive therapy. By contrast, prophylaxis more clearly controls some of the indirect effects of HCMV such as opportunistic infections. However, since preemptive therapy implies an active HCMV replication, a beneficial stimulation of a protective immunity might reduce the rate of late onset HCMV (Table 1).

Meta-analyses have been conducted on randomized, placebo controlled clinical trials and gave evidence for benefits of ganciclovir and valaciclovir for prophylaxis in solid organ recipients [26]. The meta-analyses also reported reductions in HCMV end-organ disease. Recently, a randomized controlled trial was conducted to determine whether late onset HCMV disease can better be prevented by administration of ganciclovir as prophylaxis or as preemptive therapy in renal graft recipients. It appears that routine oral prophylaxis may improve long-term graft survival for most of the patients and that pre-emptive therapy should only be considered in low risk patients (e.g. transmission from seropositive or seronegative donors to seropositive recipients), and in combination with adequate HCMV monitoring [27•].

By contrast, others still argue in favour of pre-emptive therapy, given the advancements in diagnostic assays. They suggest careful monitoring of HCMV infection in order to detect threshold levels at which antiviral therapy should be initiated, thus allowing to treat a lower number of patients for a shorter period of time [28,29••].

In summary, both prophylactic and pre-emptive therapy strategies have been used with currently approved drugs and can therefore be considered in patient groups where their effect has clearly been shown.

Table 1

Comparison of prophylactic and pre-emptive strategies for HCMV chemotherapy

Antiviral strategy		Requirements for HCMV drugs		Pros	Cons
		Safety	Potency		
Prophylactic therapy	Drug is given immediately after transplantation, before active infection/reactivation	High (long treatment period)	Medium/low (no active infection)	Better control of indirect effects of active HCMV infection (e.g. opportunistic infections, long-term graft survival)	Higher potential for resistance development Higher incidence of late onset CMV disease (problem shifted to later time point?)
Pre-emptive therapy	Drug is given after detection of active HCMV replication	Medium (limited treatment period)	High (established infection)	Treating a lower number of patients for shorter periods of time (no unnecessary use of drugs) Putative stimulation of protective immunity	Damage maybe done before HCMV is controlled High diagnostic burden

Obviously current treatment regimes have evolved on the properties of current drugs. Once better tolerated drugs become available, treatment guidelines will likely favour prophylactic therapy which is less dependent on the availability and cost issues of diagnostic procedures required for preemptive therapy.

Resistance to approved drugs

The widespread use of GCV in transplant recipients has efficiently reduced HCMV related mortality and morbidity. However, a potential risk associated with prolonged use is the emergence of drug-resistant virus strains. In fact, characteristic mutations in the HCMV genes UL97 (protein kinase) and UL54 (DNA polymerase) that confer resistance to GCV are quite common after several months of therapy [30,31]. Mutations in UL97 associated with GCV resistance do not lead to cross-resistance to other anti-HCMV drugs, as these compounds do not require activation by the UL97 kinase. By contrast, mutations in the UL54 gene may confer cross-resistance to CDV and FOS because pUL54 is the common molecular target for all current systemic HCMV drugs. Consequently, drug-resistant strains of HCMV have been found for all three compounds, and the emergence of cross-resistant strains has been described in clinical settings [32,33].

In summary, combating HCMV in the transplant setting with DNA-Polymerase inhibitors is effective and has been established for many years now. However, problems with toxicity and emergence of drug resistance underscore the need to develop new and improved antiviral drugs with novel molecular targets. This challenge has been taken up. In recent years a number of new antivirals were discovered and reached clinical development. The most advanced compound presently is Maribavir. In addition, alternative therapies are being developed and old drugs are being re-considered.

Novel antiviral approaches in the clinic

Old drugs in new clothes

CMX001 (Chimerix Inc.)

The nucleotide analogue cidofovir (CDV) is a broad-spectrum antiviral that selectively inhibits viral DNA polymerases. Although very efficient against almost all double-stranded DNA viruses, its use is limited owing to its poor oral bioavailability and its inherent nephrotoxicity. Attempts to overcome these drawbacks led to the synthesis of the CDV derivative CMX001. CMX001 (hexadecyloxypropyl-CDV; HDP-CDV) is a lipid ester prodrug of CDV that is orally active (Figure 2a) [34]. Several studies evaluating CMX001 for antiviral activity against HCMV and other DNA viruses have demonstrated excellent *in vitro* and *in vivo* efficacy [35,36]. In addition, a series of preclinical studies revealed oral bioavailability and minimal nephrotoxicity and thus paved the way for CMX001 to move into clinical trials in 2006 [34]. CMX001 is currently being developed as an oral therapeutic for the treatment of smallpox infections and HCMV (www.chimerix-inc.com/oral_cmv.php).

Artesunate (ART; Arinate®; Dafra-Pharma)

ART is an anti-malarial that is widely used in the treatment of severe malaria with encouraging pharmacological properties (Figure 2a) [37]. Chemically, ART is a semisynthetic derivative of artemisinin, the active compound of the Chinese herb *Artemisia annua*. Interestingly, in addition to its anti-malarial activity, ART also exhibits activity against viruses, including HCMV. In fact, ART demonstrates similar anti-HCMV activity to that of GCV and proved to be well tolerated [37–39]. The antiviral mode of action is not known. Initial data indicate that ART might interfere with essential cellular activation pathways [38,39]. In order to study the anti-HCMV activity of ART in a clinical setting, a small Phase III clinical trial in stem cell transplant recipients receiving pre-emptive ART therapy was started (www.clinicaltrials.gov/ct/gui/show/NCT00284687). Published results from the first patient with late drug-resistant HCMV were very promising with regard to tolerability and efficacy [40•].

New drugs—discontinued approaches

BAY-384766 (Tomeglovir®, Bayer AG)

BAY-384766 is a potent and selective inhibitor of HCMV replication and a representative of a novel non-nucleosidic class of anti-HCMV-drugs, the phenylenediamine sulfonamides (Figure 2b). It targets the viral terminase complex [41–43]. BAY-384766 prevents the cleavage of high molecular weight viral DNA concatemers to monomeric genomic lengths. Phase I trials were conducted and demonstrated a good tolerability in humans. However, by November 2000 the development of BAY-384766 was discontinued.

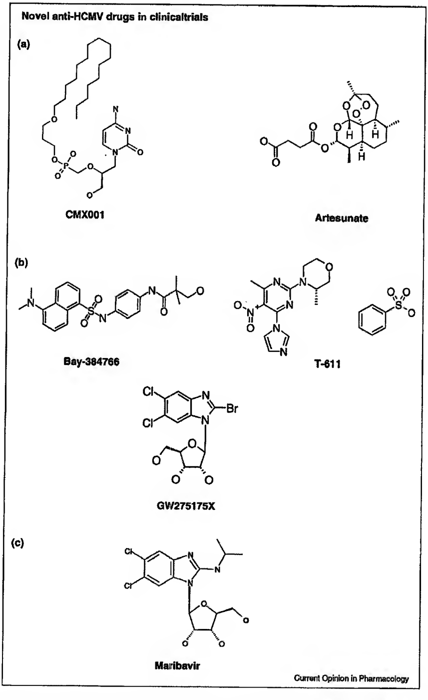
T-611 (T-0902611, Tularic Inc.)

Another novel non-nucleoside HCMV inhibitor was discovered by Tularik (now Amgen), the imidazolpyrimidine-based compound T-661 (Figure 2b). T-611 acts by inhibiting the viral primase, a key enzyme essential for the replication of the HCMV DNA. Preclinical data showed that T-661 is highly active and specific for HCMV. Moreover, the drug was less toxic in cell culture than GCV and showed a good pharmacological profile. In 2000 clinical Phase I studies showed that T-611 was orally available and well tolerated with no significant adverse effects. The compound had entered Phase II trials in 2001 in HIV/AIDS patients with HCMV infection. However, by June 2002 the programme was discontinued owing to formulation issues.

GW-275175X (175X; University of Michigan/ GlaxoSmithKline)

A third approach to the development of an orally active, less toxic HCMV antiviral has been the synthesis and evaluation of benzimidazole ribonucleosides including

Figure 2



Chemical structures of novel anti-HCMV drugs that have been evaluated in clinical trials. **(a)** Drugs with anti-HCMV activity currently marketed for an unrelated indication. **(b)** Discontinued approaches. **(c)** Anti-HCMV drug in clinical development.

the prototype BDCRB (2-Bromo-5,6-dichloro-1-β-D-ribofuranosyl-1H-benzimidazole). Although BDCRB was extensively studied *in vitro* and *in vivo*, clinical development was not pursued since the compound was metabolically unstable [44–46]. In order to obtain a more stable molecule, different analogues were synthesized including the D-ribopyranosil nucleoside GW275175X (Figure 2b). Remarkably, mode of action studies revealed that both GW275175X and its parent BDCRB act as an inhibitor of viral DNA processing in a manner similar to the chemi-

cally unrelated compound BAY-384766 [47,48]. GW275175X demonstrated good antiviral activity and showed promising results in extensive pharmacokinetic and toxicity analyses, including Phase I trials. Nevertheless, development of GW275175X was also discontinued by November 2000.

New drugs in active development

Maribavir (MBV; 1263W94; Camvia®; Viropharma)

Another analogue of the benzimidazole–ribonucleoside derivative BDCRB that has been synthesized in order to obtain a compound with better pharmaceutical properties is Maribavir (Figure 2c). The drug is a β-L-ribofuranosyl analogue of BDCRB that potently inhibits HCMV replication and is currently undergoing clinical development for the prophylaxis of HCMV infection in transplant patients. Surprisingly, MBV has a mechanism of action that is different from that of its parent compound BDCRB. It was shown that the main target of MBV is the virus-encoded protein kinase UL97, an enzyme that is involved in viral DNA synthesis and egress of viral capsids from infected cell nuclei [49–51]. However, it has also been reported that mutations in the HCMV gene UL27 (encoding for a protein of unknown function) also confer resistance against MBV, indicating that the MBV mode of action is not fully understood.

MBV was originally developed by GlaxoSmithKline for the treatment of HCMV disease in HIV patients. Development was discontinued after HAART decreased the need for an anti-HCMV compound in this patient population. Nevertheless, initial proof-of-concept studies in HIV patients indicated antiviral activity of MBV [52]. In 2003 ViroPharma licensed the drug to develop MBV for the prevention and treatment of HCMV infections in transplant recipients. A placebo-controlled dose-ranging Phase II clinical study was conducted in BMT patients and showed that maribavir prophylaxis reduced the number of patients requiring pre-emptive anti-HCMV treatment [53•]. Currently ViroPharma is conducting two pivotal international Phase III trials in patients undergoing either an allogenic stem cell or a solid organ transplantation (www.viropharma.com). These trials should reveal the performance of the drug in the intended prophylactic use.

Conclusion

HCMV remains a major problem especially in transplant recipients. Although progress in the diagnosis of HCMV infections has led to improved therapeutic strategies, treatment of HCMV induced disease suffers from the severe toxicity of the current antiviral drugs. Consequently, anti-HCMV drug discovery has been a highly active area of pharmaceutic research during the past years. Among the novel HCMV antivirals that have been described, only Maribavir is currently undergoing Phase III clinical trials. Currently each patient collective has to be considered separately in terms of the ideal treatment option and the need for an antiviral drug that is potent, safe and well tolerated in all patient groups remains.

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- •• of outstanding interest

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Letermovir (AIC246) – A Novel Drug Under Development for Prevention and Treatment of Cytomegalovirus Infections Acting via a Novel Mechanism of Action

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Abstract

Cytomegalovirus (CMV) remains an important pathogen for immunocompromised individuals, including transplant recipients, AIDS patients and newborns. It also increases morbidity and mortality in patients with temporal or partial immune suppression such as patients in intensive care units, highly active antiretroviral therapy (HAART)-treated HIV patients or patients with autoimmune disease. To date, all drugs licensed for the treatment of CMV infection and diseas e target the viral DNA polymerase. Although effective, there are several disadvantages associated with the use of these drugs, including toxicity and the emergence of drug resistance; hence, safe and improved antivirals with novel molecular targets are urgently needed. Letermovir (AIC246) belongs to a novel class of anti-CMV agents that exhibits outstanding antiviral activity and acts via a mechanism of action that is distinct from all currently approved drugs. In all clinical trials performed so far, AIC246 has been generally well tolerated in healthy subjects as well as in CMV-infected transplant patients. Efficacy was shown in a proof-of-concept trial in pre-emptively treated solid-organ transplant patients and a patient with multidrug-resistant CMV disease involving several organs. Results from a Phase IIb dose-finding trial for prophylactic use in human blood precursor cell transplanted patients are expected in early 2012.

Keywords

AIC246, Letermovir, cytomegalovirus, antiviral therapy

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The human cytomegalovirus (CMV) is a member of the herpes virus family and is widely spread in the human population with seroprevalence rates varying from 40 up to almost 100 %, depending on socioeconomic status and geographic location. After primary infection, the virus remains in the latent state in its human host for a life-time and is activated in all instances of immune incompetence or immune suppression.[1,2]

Accordingly, CMV is the most common viral pathogen in solid organ transplant recipients (i.e., kidney, heart, liver, lung and pancreas) as well as in bone marrow, cord blood and human precursor blood cell transplant recipients. CMV infections observed in these patients are either a result of reactivation of latent virus in a CMV-seropositive recipient (R+) or of a primary infection being transmitted from a seropositive donor (D+) to a seronegative recipient.[3,4]

CMV infection is a major cause of morbidity and mortality in particular during the first six months after transplantation. Furthermore, CMV is the major viral cause of birth defects and developmental disorders after birth in the developed world. Congenital CMV infections can have devastating consequences for the foetus and it is estimated that approximately 0.2–2.2 % of life births in the US are born congenitally infected. Remarkably, only 10–15 % of infected newborns have clinical symptoms at birth, including central nervous system (CNS) abnormalities in the form of deafness, vision loss, microcephaly and mental retardation. The prognosis of those children who are symptomatic at birth is very poor and about 10 % of the affected infants die as a consequence of their CMV infection. However, also in 7–15 % of those children who are infected at birth and are asymptomatic, late sequelae (progressive hearing loss, learning or behavioural difficulties) may occur months or even years later.[5,6] The impact of the consequences of congenital CMV infections on the social system has been estimated at US$1.8 billion per year.[7]

Moreover, in countries where treatment standards for HIV are low and HIV-infected patients develop AIDS, CMV is a significant pathogen in this patient population leading to severe disease and death.

However, CMV is also an immunopathogen in its own right. Recently, it has been recognised that CMV replication might be deleterious even in the absence of disease. Given this, additional patient groups where a (subclinical) CMV replication may add to morbidity and in some cases mortality are cancer patients treated with highly effective next-generation cytostatics, intensive care unit (ICU) patients and

patients undergoing aggressive immunosuppressive treatment due to an underlying autoimmune disease, e.g. colitis ulcerosa. In addition, persistent inflammation in highly active antiretroviral (HAART)-suppressed HIV patients has been attributed to CMV, causing for example cardiovascular disorders, and anti-CMV treatment was shown to reduce inflammation parameters.[8] Treatment of CMV, therefore, appears to be the next challenge in the management of HIV-infected individuals.

The current treatment of choice for CMV infections and associated disease is the nucleosidic polymerase inhibitor valganciclovir (VGCV), the oral prodrug of ganciclovir (GCV).[9,10] Neutropenia, anaemia, thrombocytopenia and a putative long-term reproductive toxicity are the most common serious side effects associated with this compound.[11] Other anti-CMV drugs, mainly used as rescue medication for patients who failed GCV therapy, are cidofovir (CDV) and foscarnet (FOS). CDV has demonstrated broad-spectrum activity against double-stranded DNA viruses including herpes viruses and acts as a competitive inhibitor of the viral DNA polymerase causing premature chain termination during viral DNA synthesis.[12] However, its clinical usefulness is limited, since the drug is slowly absorbed, poorly bioavailable and causes severe nephrotoxicity. Moreover, CDV has been found to be carcinogenic and teratogenic in animal studies.[13,14] FOS is a pyrophosphate analogue that directly binds to the pyrophosphate binding site of the viral DNA polymerase. The drug inhibits viral DNA replication by blocking the cleavage of the pyrophosphate group from the terminal nucleoside triphosphate added to the growing DNA chain. FOS does not require any intracellular chemical modification for antiviral activity.[15] The most important major adverse effect associated with FOS therapy is renal impairment.

The widespread use of GCV in transplant recipients has efficiently reduced CMV-related mortality and morbidity. However, a potential risk associated with prolonged use is the emergence of drug-resistant virus strains. In fact, characteristic mutations in the CMV genes UL97 (protein kinase required for GCV phosphorylation) and UL54 (DNA polymerase) that confer resistance to GCV can be quite common (up to approximately 10 %) after several months of therapy.[16,17] Mutations in the UL54 gene may confer cross-resistance to CDV and FOS because pUL54 is the common molecular target for all current systemic low molecular weight CMV drugs. Consequently, drug-resistant strains of CMV have been found for all three compounds (GCV, CDV and FOS) and the emergence of cross-resistant strains has been described in clinical settings.[18,19]

In summary, although combating CMV with DNA polymerase inhibitors is effective and has been established for many years, problems with toxicity, tolerability and emergence of drug resistance underscore the need to develop new and improved antiviral drugs with novel molecular targets.

Identification of Letermovir

Using high-throughput screening with a combined readout for compound toxicity and antiviral efficacy, inhibition of the viral terminase has emerged as an attractive target for novel CMV drugs.[20,21] When 3,4-dihydro-quinazoline-4-yl-acetic acid derivatives were identified as a new class of anti-CMV compounds, an extensive hit-to-lead programme including in-depth structure–activity relationship studies and pharmacological analyses led to the discovery of the low molecular weight compound Letermovir (AIC246) (see *Figure 1*). Inhibition of the viral terminase by this compound was confirmed.[22,23]

Figure 1: Chemical Structure of AIC246 (Letermovir)

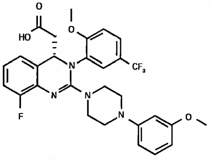

Table 1: Antiviral Efficacy of AIC246 Against Wildtype or Ganciclovir-resistant Cytomegalovirus Laboratory Strains and Clinical Isolates in Comparison with Ganciclovir

Virus	Sequence Information (UL97)	EC_{50} (µM) Ganciclovir	AIC246
Laboratory strain AD169	wt	2.4	0.005
Laboratory strain AD169	M460I	12	0.004
Clinical isolate 472	wt	5	0.002
Clinical isolate 1947	C603W	15	0.002
Clinical isolate 16415	wt	1.1	0.003
Clinical isolate 17251	C603W	14	0.006

Preclinical Virology Data

Preclinically, AIC246 antiviral potency was assessed across a number of diverse *in vitro* and cell-based assays and a mouse xenograft model of CMV infection. Across these assessments AIC246 demonstrated outstanding antiviral activity *in vitro*, surpassing the current gold standard by more than 400-fold with respect to 50 % effective concentration (EC_{50}) values (4.5 nM versus 2 µM) and by more than 2,000-fold with respect to EC_{90} values (6.1 nM versus 14.5 µM), without showing an apparent cytotoxic effect. Interestingly, the antiviral activity of AIC246 is characterised by a very steep dose-response curve, which accounts for the only slight differences between EC_{50} and EC_{90} values.[22] Another intriguing characteristic of AIC246 is its stable antiviral response even under increasing viral loads. Cell culture experiments revealed that the efficacy of AIC246 is only moderately influenced by a 333-fold titre increase in virus inoculum, leading to an only three-fold increase in the respective EC_{50}. In contrast, GCV EC_{50} values increase up to six-fold in the µM range in parallel experiments. These results suggest that AIC246 is sufficiently potent to combat infections associated with high virus titres.[22]

Importantly, AIC246 efficacy is not restricted to CMV laboratory strains but it also inhibits CMV clinical isolates including GCV-resistant strains with similar efficacy. Experiments performed to assess the potential utility of the drug in the treatment of resistant infections evaluated the AIC246 efficacy against GCV-resistant mutants and a panel of different CMV clinical isolates. As summarised in *Table 1*, all virus strains tested were comparably sensitive to AIC246, with EC_{50} values ranging from 2 to 6 nM. Mechanistically, this coverage of resistant viruses is due to the novel mechanism of action of AIC246, which is unique in relation to the currently approved CMV antivirals, in that AIC246 interferes with the activities of the viral terminase proteins rather than with that of the viral DNA polymerase. The viral terminase is an enzyme complex

Table 2: Clinical Effect of AIC246 Treatment in Patients Infected with Ganciclovir-resistant Viruses

		Response to Antiviral Therapy	
Virus	Amino Acid Mutations (UL54/UL97)	Ganciclovir, Foscarnet, Cidofovir	AIC246
123-001[25]	UL54: E756D, A809V, K513N, S655L, N685S, V759M, A885T, N898D UL97: L595W	-	+
Case report[26]	UL54: A987G UL97: A594T, C603W	-	+

that plays a key role in cleavage and packaging of CMV progeny DNA into capsids. This mechanism of action is expected to offer an advantage over current therapies given its antiviral efficacy without the target-related toxicity seen for the marketed nucleosidic anti-CMV drugs, since mechanism-based side effects are unlikely due to the lack of a mammalian counterpart of the viral terminase enzyme. In addition, the different mode of action of AIC246 should provide new treatment options for patients infected with resistant virus strains for which no effective therapy is currently available.[23]

Phase I Data

During clinical development, the safety, tolerability and pharmacokinetic properties of AIC246 have been monitored in nine Phase I trials so far. After single oral dosing, absorption occurs rapidly with median t_{max} of 1.5 hours and a mean terminal elimination half-life of 10 hours. To date, AIC246 has been administered to more than 230 healthy male and female subjects, either as single doses or repeated doses for up to 14 days. In all trials, AIC246 was generally well tolerated as no dose-dependent adverse events occurred and no effects on safety laboratory parameters, vital signs and electrocardiogram (ECG) parameters were detected.[24]

Phase II Data

In an open-label proof-of-concept trial 27 transplanted patients with positive CMV viraemia were enrolled and treated pre-emptively with daily doses of 80 mg AIC246 for 14 days. The reduction in viral CMV DNA titres in kidney-transplanted patients was similar compared with the local standard treatment of the investigational site and no patient developed CMV disease under therapy. Also, the efficacy of 1 x 80 mg and 2 x 40 mg per day was comparable, indicating that once-daily dosing is feasible. Notably, in this trial, AIC246 was also used to successfully treat a patient harbouring a multi-resistant CMV strain for the marketed anti-CMV drugs GCV, CDV and FOS, confirming the resistance-breaking potential seen in the *in vitro* studies (see *Table 2*). Overall, AIC246 co-administration with immunosuppressive drugs in patients did not result in a need for major adjustments of the co-administered immunosuppressants. Moreover, no tolerability or safety issues related to AIC246 treatment occurred during AIC246 treatment.[25]

Emergency Use of AIC246

AIC246 was successfully used to treat a lung-transplanted patient with refractory multidrug resistant CMV disease involving three organs (lung, colon and eye) under an US Emergency Investigational New Drug (EIND) application (see *Table 2*). Resolution of viraemia was reached after 28 days on treatment. Clinical and radiological resolution of CMV pneumonitis, retinitis and colitis was achieved by 33 days of AIC246 therapy. Patient plasma quantitative CMV polymerase chain reaction (PCR) remained undetectable over a four-month follow-up period. No tolerability or safety issues related to AIC246 treatment occurred during the treatment period.[26]

Summary

AIC246 is derived from a novel chemical class and acts via a novel mechanism of action compared with drugs on the market and in development. This novel drug is highly active against the human CMV and is resistance breaking versus viruses resistant to all currently used compounds. Phase I trials demonstrated that AIC246 was generally well tolerated and showed a high and long-lasting exposure in human subjects, allowing once-daily dosing. Moreover, proof of concept for efficacy was shown in a Phase IIa trial in solid-organ-transplanted patients treated pre-emptively and in a patient infected with a multidrug-resistant virus suffering from multi-organ CMV disease. As such, AIC246 appears to be a promising new drug with resistance-breaking properties suitable for treatment and prevention of CMV infection and disease. A Phase IIb multicentre, randomised, double-blind, placebo-controlled dose escalation trial of AIC246 for prevention of CMV infection/reactivation in haematopoietic stem cell transplant recipients has enrolled all patients (clinicaltrials.gov identifier: NCT01063829) and results should be available in early 2012. ■

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